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Selection of a Surrogate Agent (Fluconazole or Voriconazole) for Initial Susceptibility Testing of Posaconazole against *Candida* spp.: Results from a Global Antifungal Surveillance Program▽

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There are currently no FDA-approved broth microdilution antifungal susceptibility testing products or interpretive breakpoints for susceptibility testing of the new triazole posaconazole. Fluconazole and voriconazole are in the same triazole class as posaconazole, have CLSI-approved interpretive MIC breakpoints, and are available on some commercially available MIC panels. We investigated whether one or both of these agents may be useful as a surrogate marker for posaconazole susceptibility. Fluconazole, voriconazole, and posaconazole MIC results for 10,807 isolates of *Candida* spp. were analyzed to validate a potential surrogate marker for posaconazole activity against indicated species. For illustrative purposes, we applied the voriconazole MIC breakpoints to posaconazole (susceptible, ≤1 μg/ml; susceptible dose dependent, 2 μg/ml; resistant, ≥4 μg/ml) and compared these MIC results and categorical interpretations with those of fluconazole and voriconazole by using regression statistics and categorical agreement. For all 10,807 isolates, the absolute categorical agreement was 91.1% (0.1% very major errors [VME], 1.2% major errors [ME], and 7.6% minor errors [M]) using fluconazole as the surrogate marker and 97.7% (0.3% VME 0.1% ME, and 1.9% M) using voriconazole as the surrogate. The results with fluconazole improved to a categorical agreement of 93.7% (0.1% VME, 0.2% ME, and 6.0% M) when results for *Candida krusei* (not indicated for fluconazole testing) were omitted. Either fluconazole or voriconazole MIC results may serve as surrogate markers to predict the susceptibility of *Candida* spp. to posaconazole.

Posaconazole is a new triazole antifungal agent with broad-spectrum activity against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., and other opportunistic and endemic fungal pathogens (6, 7, 9, 13, 18, 24, 28, 36, 37, 41). The activity of posaconazole against *Candida* spp. has been documented in vitro by the broth microdilution (BMD) (6, 24, 28, 41), disk diffusion, and Etest (AB BIODISK, Solna, Sweden) methods (8, 44). Although posaconazole is active against isolates of *Candida* spp. with decreased susceptibility to fluconazole, evidence of cross-resistance has been demonstrated, especially with fluconazole-resistant strains of *C. glabrata* (18, 24, 25, 28, 41).

Posaconazole has therapeutic indications for salvage therapy of invasive aspergillosis, fusariosis, chromoblastomycosis, and coccidioidomycosis (1, 39, 46, 49). It is also indicated as first-line therapy for oropharyngeal candidiasis (OPC) (45, 48) and for prophylaxis of invasive fungal infections in neutropenia and in hematopoietic stem cell transplant recipients (5, 47). Although the emergence of fungi with reduced susceptibility to posaconazole was not detected during the treatment of OPC (45, 48) or the invasive fungal infection prophylaxis study periods (5, 47), the development of resistance remains a concern with both prophylaxis and OPC therapy and warrants further investigation.

Although both agar-based and BMD antifungal susceptibility testing methods have been validated for testing posaconazole against *Candida* (8), the immediate lack of commercial antifungal susceptibility testing products and interpretive breakpoints for susceptibility testing of this agent requires a surrogate marker agent to assist microbiologists and clinicians in the correct categorization of potentially indicated species of *Candida* (15, 16, 30, 35). The facts that both fluconazole and voriconazole are in the same triazole class as posaconazole, have Clinical and Laboratory Standards Institute (CLSI)-approved MIC interpretive breakpoints (32, 33), and are available on some commercially available MIC panels (10, 19, 29, 34, 38) suggest that one or both of these agents may be useful as a surrogate marker for posaconazole susceptibility.

The purposes of the present study were to provide further documentation of cross-resistance among fluconazole, voriconazole, and posaconazole and to examine the usefulness of both fluconazole and voriconazole as surrogate markers for evaluating posaconazole susceptibility in *Candida* spp. by using a large database of MIC results compiled in the course of global antifungal surveillance studies (26–28, 35).

MATERIALS AND METHODS

Organisms. A total of 10,807 clinical isolates of *Candida* spp. submitted by more than 100 medical centers worldwide from January 2001 to December 2006 were tested. The collection included 5,827 *Candida albicans* isolates, 1,542 *Candida parapsilosis* isolates, 1,517 *Candida glabrata* isolates, 1,198 *Candida tropicalis* isolates, 305 *Candida krusei* isolates, 138 *Candida guilliermondii* isolates, 133 *Candida lusitaniae* isolates, 51 *Candida kefyr* isolates, 31 *Candida pelliculosa* isolates, 19 *Candida famata* isolates, 13 *Candida rugosa* isolates, 12 *Candida dubliniensis* isolates, 12 *Candida lipolytica* isolates, and 8 *Candida zeylanoides* isolates. All of these isolates were incident isolates from individual patients and were obtained from blood or other normally sterile body fluids. Isolates were

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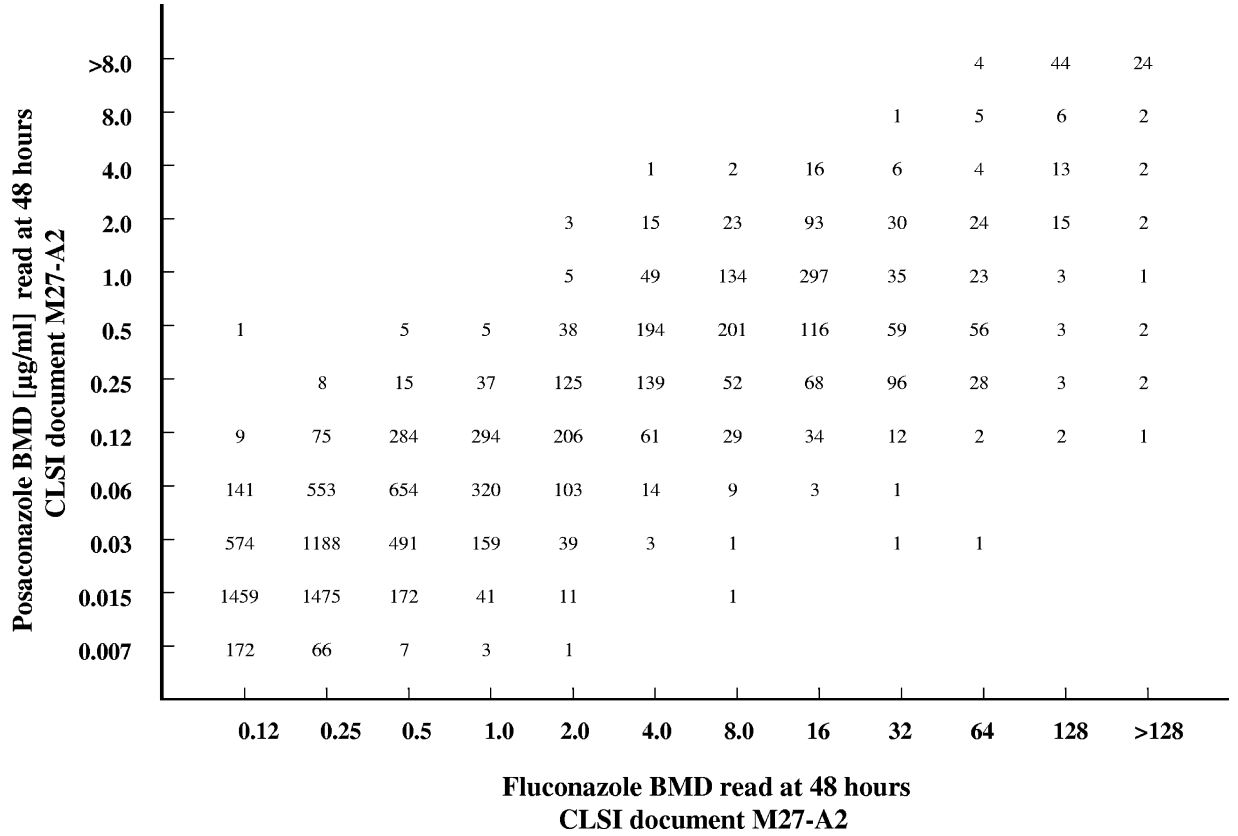

FIG. 1. Scattergram comparing fluconazole and posaconazole MICs for 10,807 strains of *Candida* spp. An excellent correlation was observed ($r = 0.88$; $y = 0.7425x - 1.3048$).

identified by using Vitek and API yeast identification systems (bioMérieux, Inc., Hazelwood, MO) and were supplemented by conventional methods as needed (14). Isolates were stored as water suspensions until they were used. Prior to testing, each isolate was passaged at least twice on potato dextrose agar (Remel, Lenexa, KS) and CHROMagar (Hardy Laboratories, Santa Maria, CA) to ensure purity and viability.

Susceptibility testing. Reference antifungal susceptibility testing of all isolates was performed by BMD as described by the CLSI (20). Reference powders of fluconazole (Pfizer), voriconazole (Pfizer), and posaconazole (Schering-Plough) were obtained from their respective manufacturers.

MIC interpretive criteria for fluconazole and voriconazole were those published by Pfaller et al. (32, 33) and the CLSI (20). Breakpoints were as follows: susceptible (S), ≤8 μg/ml (fluconazole) and ≤1 μg/ml (voriconazole); susceptible dose dependent (SDD), 16 to 32 μg/ml (fluconazole) and 2 μg/ml (voriconazole); resistant (R), ≥64 μg/ml (fluconazole) and ≥4 μg/ml (voriconazole). Posaconazole has not been assigned interpretive breakpoints by the CLSI. For purposes of comparison, we applied the MIC breakpoints listed above for voriconazole, i.e., ≤1 μg/ml (S), 2 μg/ml (SDD), and ≥4 μg/ml (R).

Analysis of results. All MICs (expressed in micrograms per milliliter) of fluconazole and voriconazole were directly compared with those of posaconazole by using regression statistics and a scattergram (Fig. 1 and 2). Acceptable error limits used in this comparison were those cited by the CLSI and by other authors (4, 11, 16).

The definitions of errors used in this analysis were as follows: a very major error (VME), or a false-susceptible error, was a result of S for the surrogate marker fluconazole or voriconazole and a result of R for posaconazole; a major error (ME), or a false-resistant error, was a result of R for fluconazole or voriconazole and a result of S for posaconazole; and minor errors occurred when the result for one of the agents was S or R and that for the other agent was SDD. In general, for an agent to be considered a reliable surrogate, the VME rate should be ≤1.5% of all results and the absolute categorical agreement between methods should be ≥90% (4, 11, 16).

RESULTS AND DISCUSSION

Table 1 depicts the MIC profiles and percentages of S and R for fluconazole, voriconazole, and posaconazole determined for 10,807 strains of *Candida* spp. by using CLSI-validated BMD methods (20). Overall, 9,667 isolates (89.5%) were S, 868 (8.0%) were SDD, and 272 (2.5%) were categorized as R to fluconazole. Likewise, 10,656 isolates (98.6%) were S, 55 (0.5%) were SDD, and 92 (0.9%) were categorized as R to voriconazole. By comparison, 10,472 isolates (96.9%) were S, 205 (1.9%) were SDD, and 130 (1.2%) were R to posaconazole at MIC breakpoints of ≤1 μg/ml, 2 μg/ml, and ≥4 μg/ml, respectively. The modal MIC for posaconazole was 0.015 μg/ml, compared to 0.25 μg/ml for fluconazole and 0.007 μg/ml for voriconazole. Decreased potencies for all three agents were observed among *C. glabrata* (modal MICs of 1 μg/ml, 16 μg/ml, and 0.25 μg/ml for posaconazole, fluconazole, and voriconazole, respectively) and *C. krusei* isolates were susceptible to both posaconazole and voriconazole at ≤1 μg/ml. Aside from *C. glabrata* and *C. krusei*, decreased susceptibility (<90%) to fluconazole was noted among isolates of *C. rugosa* (61.5% S) and *C. zeylanoides* (87.5% S). *C. glabrata* (79.6% S), *C. pelliculosa* (58.1% S), and *C. zeylanoides* (87.5% S) showed decreased susceptibility to posaconazole, whereas decreased susceptibility to voriconazole was only seen with *C. zeylanoides* (87.5% S).

The extent of cross-resistance between fluconazole and

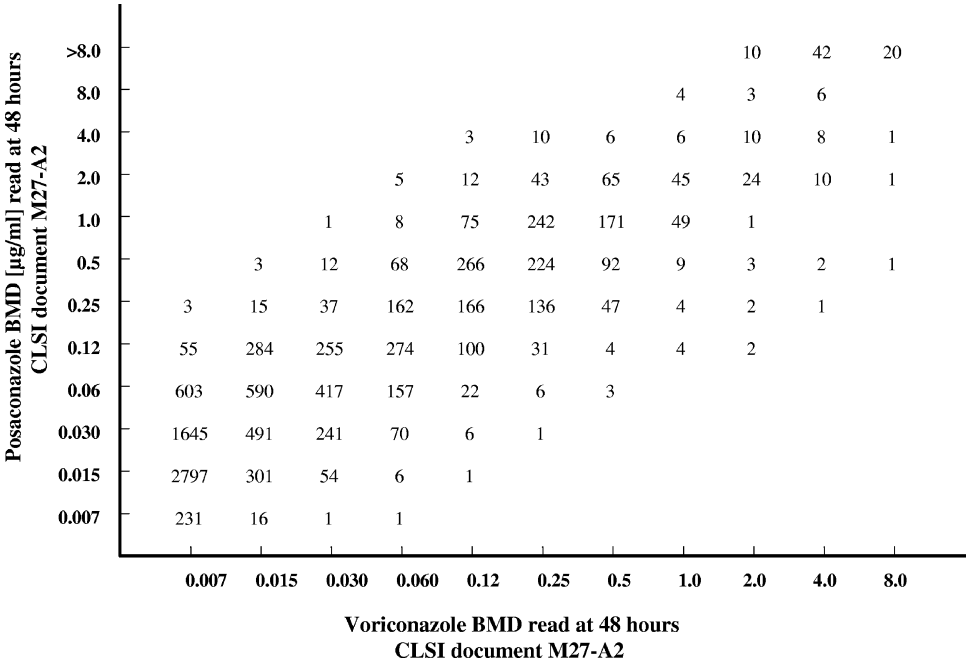

FIG. 2. Scattergram comparing voriconazole and posaconazole MICs for 10,803 strains of *Candida* spp. An excellent correlation was observed ($r = 0.89$; $y = 0.8964x + 1.7269$).

posaconazole may be seen more clearly in Table 2 and Fig. 1. As was seen previously in comparisons of fluconazole and ravuconazole (30) and fluconazole and voriconazole (35), there was a strong positive correlation ($r = 0.88$) between posaconazole and fluconazole (Fig. 1). More than 99% (99.5%) of the fluconazole-susceptible isolates were susceptible to posaconazole, as were 83.2% of the fluconazole-SDD isolates (Table 2). Among 272 fluconazole-resistant isolates, 127 (46.7%) were susceptible, 41 (15.1%) were SDD, and 104 (38.2%) were resistant to posaconazole.

It should be noted that none of the fluconazole-resistant isolates of *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were resistant to posaconazole, whereas 102 (70%) of the fluconazole-resistant *C. glabrata* isolates were also resistant to posaconazole (Table 2). This is consistent with the predominant resistance mechanisms seen with these species. Posaconazole is known to bind more extensively to the target enzyme, 14α-demethylase, of *C. albicans* than does fluconazole, due in part to the presence of a long hydrophobic side chain that serves to stabilize the binding of posaconazole to the target, making it less susceptible to the effect of point mutations in the *ERG11* gene (3, 50). Indeed, Li et al. (17) demonstrated that isolates of *C. albicans*, from a patient with OPC, that were resistant to fluconazole and voriconazole but susceptible to posaconazole all had the same five missense mutations in *ERG11* that specifically reduced the binding of fluconazole and voriconazole to the target enzyme. Furthermore, subsequent isolates obtained during the course of posaconazole therapy had all acquired an additional mutation, leading to the disruption of the binding of the posaconazole side chain within the hydrophobic channel of the enzyme (17). Thus, in order for *C. albicans* to exhibit resistance to posaconazole, there is a requirement for mutational events affecting the target enzyme that are over and above those necessary to produce resistance to fluconazole and voriconazole.

In contrast, the primary mechanism of resistance to azoles in *C. glabrata* involves upregulation of the genes encoding the CDR efflux pumps (2, 43). All of the azoles, including posaconazole, serve as substrates for the CDR pumps (42), and as a result, cross-resistance to all azoles is a common feature in fluconazole-resistant *C. glabrata* isolates (43).

The CLSI does not recommend that laboratories test *C. krusei* against fluconazole, given its poor clinical response to this agent and the fact that the "intrinsic" resistance manifested by this species may be underrepresented by the in vitro results (12, 20, 30–32, 35, 40). In contrast, posaconazole appears quite active against *C. krusei* (302 [99%] of 305 isolates were susceptible at an MIC of ≤1 μg/ml [Tables 1 and 2]). It is likely that, as with voriconazole (12), posaconazole binds much more tightly to the target enzyme of *C. krusei* than does fluconazole. As noted previously for both ravuconazole (30) and voriconazole (35), it appears that susceptibility of *C. krusei* to posaconazole is predictable and testing of this drug-organism combination may not be necessary. When the *C. krusei* results are removed from the total, we again find that 99.5% of the fluconazole-susceptible isolates and 78.4% of the fluconazole-SDD isolates are susceptible to posaconazole but that only 15% of the fluconazole-resistant isolates are susceptible to posaconazole (data not shown).

When the fluconazole test result category (S, SDD, or R) was used to predict the posaconazole category, the absolute categorical agreement between test results was 91.1%, with 0.1% VME (false-susceptible error), 1.2% ME (false-resistant error), and a 7.6% M rate (Table 3). Given the fact that the fluconazole results clearly do not predict the susceptibility of *C. krusei* to posaconazole (Tables 1 and 2), we have omitted

TABLE 1. Comparative in vitro susceptibilities of more than 10,000 clinical isolates of *Candida* species to fluconazole, voriconazole, and posaconazole determined by CLSI methods

Species	Antifungal agent	No. of isolates tested	MIC (μg/ml)			% S	% R
			Range	50% of isolates	90% of isolates		
C. albicans	Fluconazole	5,827	0.12–>128	0.25	0.5	99.3	0.1
	Voriconazole	5,826	0.007–4	0.007	0.015	99.9	<0.1
	Posaconazole	5,827	0.007–2	0.015	0.06	99.9	0.0
C. parapsilosis	Fluconazole	1,542	0.12–>128	0.5	2.0	96.2	0.6
	Voriconazole	1,541	0.007–8	0.015	0.06	99.6	0.1
	Posaconazole	1,542	0.007–1	0.06	0.12	100	0.0
C. glabrata	Fluconazole	1,517	0.25–>128	8.0	32	53.1	9.6
	Voriconazole	1,516	0.007–8	0.25	1.0	91.1	5.7
	Posaconazole	1,517	0.03–>8	1.0	2.0	79.6	8.3
C. tropicalis	Fluconazole	1,198	0.12–128	0.5	2.0	99.2	0.2
	Voriconazole	1,197	0.007–8	0.03	0.06	99.8	<0.1
	Posaconazole	1,198	0.015–2	0.06	0.12	99.9	0.0
C. krusei	Fluconazole	305	8–>128	32	64	1.3	34.1
	Voriconazole	305	0.07–4	0.25	0.5	99.7	0.3
	Posaconazole	305	0.03–4	0.25	1.0	99.0	0.3
C. guilliermondii	Fluconazole	138	0.5–32	4.0	8.0	92.8	0.0
	Voriconazole	138	0.015–2	0.06	0.12	99.3	0.0
	Posaconazole	138	0.015–2	0.25	0.5	97.1	0.0
C. lusitaniae	Fluconazole	133	0.12–64	0.5	1.0	97.7	1.5
	Voriconazole	133	0.007–1	0.007	0.015	100	0.0
	Posaconazole	133	0.015–1	0.03	0.12	100	0.0
C. kefyr	Fluconazole	51	0.12–1	0.25	1.0	100	0
	Voriconazole	51	0.007–0.06	0.007	0.015	100	0.0
	Posaconazole	51	0.015–0.5	0.06	0.25	100	0.0
C. pelliculosa	Fluconazole	31	1–8	4.0	8.0	100	0.0
	Voriconazole	31	0.03–0.5	0.12	0.25	100	0.0
	Posaconazole	31	0.25–4	1.0	2.0	58.1	6.5
C. famata	Fluconazole	19	0.5–16	2.0	8.0	94.7	0.0
	Voriconazole	19	0.007–1	0.06	0.25	100	0.0
	Posaconazole	19	0.03–2	0.25	1.0	94.7	0.0
C. rugosa	Fluconazole	13	0.5–16	4.0	16	61.5	0.0
	Voriconazole	13	0.015–0.25	0.06	0.25	100	0.0
	Posaconazole	13	0.03–0.5	0.06	0.25	100	0.0
C. dubliniensis	Fluconazole	12	0.12–8	0.25	0.25	100	0.0
	Voriconazole	12	0.007–0.03	0.007	0.03	100	0.0
	Posaconazole	12	0.015–0.12	0.03	0.06	100	0.0
C. lipolytica	Fluconazole	12	1–64	4.0	8.0	91.7	8.3
	Voriconazole	12	0.015–1	0.06	0.12	100	0.0
	Posaconazole	12	0.12–4	0.5	1.0	91.7	8.3
C. zeylanoides	Fluconazole	8	0.12–>128	0.5		87.5	12.5
	Voriconazole	8	0.007–4	0.007		87.5	12.5
	Posaconazole	8	0.007–2	0.06		87.5	0.0
All *Candida*	Fluconazole	10,807	012–>128	0.25	16	89.5	2.5
	Voriconazole	10,803	0.007–8	0.015	0.25	98.6	0.9
	Posaconazole	10,807	0.007–>8	0.03	1.0	96.9	1.2

these results from the analysis, with a resulting improvement in categorical agreement (93.7%) and a decrease in both ME (0.2%) and M (6.0%) (Table 3). These results are virtually the same as those reported previously using fluconazole results to predict the susceptibility of *Candida* spp. to ravuconazole (30) and to voriconazole (35).

An absolute categorical agreement of 90% or better (range, 96.2 to 100%) was observed for all of the species tested, except

TABLE 2. In vitro activity of posaconazole against 10,807 clinical isolates of *Candida* species stratified by fluconazole susceptibility category

Species	Fluconazole susceptibility category (no. of isolates tested)	No. for which posaconazole MIC (μg/ml) was:											
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
C. albicans	S (5,789)	247	2,987	1,862	620	54	13	4	1	1			
	SDD (30)			1	1	2	10	10	6				
	R (8)					2	3	2	1				
C. parapsilosis	S (1,483)	1	30	201	625	544	54	27	1				
	SDD (49)				2	14	23	9	1				
	R (10)					1	6	3					
C. glabrata	S (805)			3	12	45	173	381	166	24	1		
	SDD (567)				1		9	94	318	122	22	1	
	R (145)						1		4	38	17	13	72
C. tropicalis	S (1,188)		118	315	459	237	55	4					
	SDD (8)					2	3	2	1				
	R (2)					1				1			
C. krusei	S (5)		1				2		2				
	SDD (197)					25	113	54	4	1			
	R (103)			1		1	22	55	22	1	1		
C. guilliermondii	S (128)		1	4	5	38	58	15	3	4			
	SDD (10)						4	6					
	R (0)												
C. lusitaniae	S (130)		16	51	46	12	4		1				
	SDD (1)								1				
	R (2)						1	1					
C. kefyr	S (51)		2	10	14	19	5	1					
	SDD (0)												
	R (0)												
C. pelliculosa	S (31)						4	4	10	11	2		
	SDD (0)												
	R (0)												
C. famata	S (18)			1	2	6	5	2	1	1			
	SDD (1)								1				
	R (0)												
C. rugosa	S (8)			3	5								
	SDD (5)					3	2						
	R (0)												
C. dubliniensis	S (12)		2	4	5	1							
	SDD (0)												
	R (0)												
C. lipolytica	S (11)					1	1	6	3				
	SDD (0)												
	R (1)										1		
C. zeylanoides	S (7)	1	1	1	1	1	2						
	SDD (0)												
	R (1)									1			
All *Candida*	S (9,667)	249	3,159	2,455	1,794	958	376	444	188	41	3		
	SDD (868)			1	4	46	164	175	332	123	22	1	
	R (272)			1		5	33	61	27	41	19	13	72

C. glabrata (66.2%), *C. krusei* (2.3%), *C. guilliermondii* (89.9%), *C. pelliculosa* (58.1%), *C. famata* (89.5%), *C. rugosa* (61.5%), and *C. zeylanoides* (87.5%).

As seen with *C. krusei*, the fluconazole results also underestimate the activity of posaconazole against *C. glabrata*, *C. guilliermondii*, *C. famata*, and *C. rugosa* (Tables 2 and 3). Between 94 and 100% of the fluconazole-susceptible isolates of these four species were also susceptible to posaconazole (Table 2). Likewise, 74% of the *C. glabrata* isolates and all of the *C. guilliermondii*, *C. famata*, and *C. rugosa* isolates that were SDD

TABLE 3. Absolute categorical agreement and error rates when the fluconazole result was used to predict the posaconazole susceptibility of *Candida* spp.

Species	No. of isolates tested	% Agreement	% VME	% ME	% M
All *Candida*	10,807	91.1 (93.7)[a]	0.1 (0.1)[a]	1.2 (0.2)[a]	7.6 (6.0)[a]
C. albicans	5,827	99.3	0.0	0.1	0.6
C. parapsilosis	1,542	96.2	0.0	0.6	3.2
C. glabrata	1,517	66.2 (86.0)[b]	0.1 (1.6)[b]	0.3 (0.3)[b]	33.4 (12.1)[b]
C. tropicalis	1,198	99.2	0.0	0.1	0.7
C. krusei	305	2.3	0.0	33.1	64.6
C. guilliermondii	138	89.9 (97.1)[b]	0.0	0.0	10.1 (2.9)[b]
C. lusitaniae	133	97.7	0.0	1.5	0.8
C. kefyr	51	100	0.0	0.0	0.0
C. pelliculosa	31	58.1	6.4	0.0	35.5
C. famata	19	89.5 (94.7)[b]	0.0	0.0	10.5 (5.3)[b]
C. rugosa	13	61.5 (100)[b]	0.0	0.0	38.5 (0.0)[b]
C. dubliniensis	12	100	0.0	0.0	0.0
C. lipolytica	12	100	0.0	0.0	0.0
C. zeylanoides	8	87.5	0.0	0.0	12.5

[a] The value in parentheses is based on the results for all of the *Candida* species minus *C. krusei* (10,502 isolates).
[b] The value in parenthesis was obtained by using the following categories for fluconazole: susceptible, MIC of ≤32 μg/ml (S and SDD combined); resistant, MIC of ≥64 μg/ml.

to fluconazole were susceptible to posaconazole. As was done in previous studies with ravuconazole (30) and voriconazole (35), it is possible to improve the ability of the fluconazole MIC test to predict the susceptibility of *C. glabrata*, *C. guilliermondii*, *C. famata*, and *C. rugosa* to posaconazole by combining the fluconazole S and SDD categories and using fluconazole MICs of ≤32 μg/ml to identify posaconazole-susceptible isolates and MICs of ≥64 μg/ml to identify posaconazole resistance. Using this criterion, the categorical agreement for *C. glabrata* improves to 86.0%, with 1.6% VME, 0.3% ME, and 12.1% M. Similarly, the categorical agreements for *C. famata*, *C. guilliermondii*, and *C. rugosa* improve to 94.7%, 97.1%, and 100%, respectively. Applying this modified criterion to the entire collection of isolates (minus *C. krusei*) results in an overall categorical agreement of 97.6%, with 0.2% VME and 0.2% ME.

A similar approach can be taken to assess the extent of cross-resistance between voriconazole and posaconazole and to determine the ability of voriconazole to act as a surrogate marker for the susceptibility of *Candida* spp. to posaconazole. Similar to that seen in the comparison of fluconazole and posaconazole, there was a strong positive correlation ($r = 0.89$) between posaconazole and voriconazole MICs (Fig. 2). Overall, the essential agreement (MIC ± 2 dilutions) was 88% (MIC ± 1 dilution was 58%) (Fig. 2), indicating the comparable potencies of these extended-spectrum triazoles against a large collection of *Candida* isolates. As seen with fluconazole, 98% of the voriconazole-susceptible isolates were susceptible to posaconazole. Among 55 voriconazole-SDD isolates, 8 (14%) were susceptible, 24 (44%) were SDD, and 23 (42%) were resistant to posaconazole. Likewise, among 92 voriconazole-resistant isolates (86 of which were *C. glabrata*), 4 (4.3%) were susceptible, 11 (12%) were SDD, and 77 (83.7%) were resistant to posaconazole. Thus, 98% of the voriconazole-susceptible and 92% of the voriconazole-nonsusceptible (SDD plus R) isolates were susceptible and nonsusceptible, respectively, to posaconazole. It is notable that the only voriconazole-nonsusceptible isolates that were resistant to posaconazole were isolates of *C. glabrata*: 48% of voriconazole-SDD isolates and 89.5% of voriconazole-resistant isolates of *C. glabrata* were resistant (MIC, ≥4 μg/ml) to posaconazole (Table 4). Importantly, none of the voriconazole-resistant isolates of *C. glabrata* were susceptible (MIC ≤1 μg/ml) to posaconazole. These findings are consistent with the known mechanism of azole resistance among isolates of *C. glabrata*.

When the voriconazole test result category (S, SDD, or R) was used to predict the posaconazole category, the absolute categorical agreement between test results was 97.7%, with 0.3% VME, 0.1% ME, and 1.9% M rates (Table 5). Among the 14 species of *Candida* tested, the categorical agreement was 90% or better (range, 91.7% to 100%) for all species except *C. glabrata* (86.2%), *C. pelliculosa* (58%), and *C. zeylanoides* (87.5%). For the most part, the discrepancies in category results involving these species were minor errors, although unacceptably high VME rates were seen with *C. pelliculosa* (6.5%) and *C. lipolytica* (8.3%). Aside from these two uncommon species, voriconazole accurately predicted susceptibility and resistance to posaconazole among *Candida* spp.

The results of this study clearly demonstrate the extent of cross-resistance among posaconazole, fluconazole, and voriconazole. Although rare, isolates of *C. albicans* that are resistant to either fluconazole or voriconazole may be susceptible to posaconazole, depending on the number and locations of target enzyme mutations and the expression of CDR efflux pumps. The latter resistance mechanism, however, ensures virtually complete cross-resistance among the triazoles with isolates of *C. glabrata*. Only 3% of the fluconazole-resistant isolates and none of the voriconazole-resistant isolates of *C. glabrata* were susceptible to posaconazole. Conversely, there is no cross-resistance between fluconazole and either posaconazole or voriconazole among isolates of *C. krusei*, a species that is predictably susceptible to these two extended-spectrum triazoles.

The strategy of using class representatives or surrogate markers to predict susceptibility or resistance to other agents in the same class has been used for decades in antibacterial susceptibility testing to develop practical alternatives for the

TABLE 4. In vitro activity of posaconazole against 10,803 clinical isolates of *Candida* species stratified by voriconazole susceptibility category

Species	Voriconazole susceptibility category (no. of isolates tested)	No. for which posaconazole MIC (μg/ml) was:											
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
C. albicans	S (5,824)	247	2,988	1,861	621	57	26	15	8	1			
	SDD (1)					1							
	R (1)							1					
C. parapsilosis	S (1,535)	1	29	201	627	558	81	36	2				
	SDD (4)					1	1	2					
	R (2)						1	1					
C. glabrata	S (1,382)			3	13	45	183	475	487	151	21	4	
	SDD (48)								1	24	10	3	10
	R (86)									9	9	6	62
C. tropicalis	S (1,195)		118	314	459	240	57	6	1				
	SDD (1)						1						
	R (1)									1			
C. krusei	S (304)		1	1		26	137	108	28	2	1		
	SDD (0)												
	R (1)							1					
C. guilliermondii	S (137)		1	4	5	38	62	20	3	4			
	SDD (1)							1					
	R (0)												
C. lusitaniae	S (133)		16	51	46	12	5	1	2				
	SDD (0)												
	R (0)												
C. kefyr	S (51)		2	10	14	19	5	1					
	SDD (0)												
	R (0)												
C. pelliculosa	S (31)						4	4	10	11	2		
	SDD (0)												
	R (0)												
C. famata	S (19)			1	2	6	5	2	2	1			
	SDD (0)												
	R (0)												
C. rugosa	S (13)			3	5	3	2						
	SDD (0)												
	R (0)												
C. dubliniensis	S (12)		2	4	5	1							
	SDD (0)												
	R (0)												
C. lipolytica	S (12)					1	1	6	3		1		
	SDD (0)												
	R (0)												
C. zeylanoides	S (7)	1	1	1	1	1	2						
	SDD (0)												
	R (1)									1			
All *Candida*	S (10,656)	249	3,159	2,454	1,798	1,007	570	674	546	170	25	4	
	SDD (55)					2	2	3	1	24	10	3	10
	R (92)						1	3		11	9	6	62

microbiology laboratory when specific diagnostic susceptibility testing reagents are limited or unavailable (4, 15, 16, 21–23). Given the lack of FDA-approved testing systems and CLSI/FDA breakpoints for posaconazole, the approach described in the present study provides a useful strategy for laboratories in the effort to optimize antifungal therapy of candidal infections.

As was shown previously for ravuconazole (30) and for voriconazole (35), fluconazole functioned well as a surrogate

TABLE 5. Absolute categorical agreement and error rates when the voriconazole result was used to predict the posaconazole susceptibility of *Candida* spp.

Species	No. of isolates tested	% Agreement	% VME	% ME	% M
All *Candida*	10,803	97.7	0.3	0.1	1.9
C. albicans	5,826	99.9	0.0	0.05	0.05
C. parapsilosis	1,541	99.6	0.0	0.1	0.3
C. glabrata	1,516	86.2	1.7	0.0	12.1
C. tropicalis	1,197	99.8	0.0	0.0	0.2
C. krusei	305	98.7	0.3	0.3	0.7
C. guilliermondii	138	96.4	0.0	0.0	3.6
C. lusitaniae	133	100	0.0	0.0	0.0
C. kefyr	51	100	0.0	0.0	0.0
C. pelliculosa	31	58.0	6.5	0.0	35.5
C. famata	19	94.7	0.0	0.0	5.3
C. rugosa	13	100	0.0	0.0	0.0
C. lipolytica	12	91.7	8.3	0.0	0.0
C. dubliniensis	12	100	0.0	0.0	0.0
C. zeylanoides	8	87.5	0.0	0.0	12.5

marker for posaconazole when applied to this collection of clinically significant isolates of *Candida* spp. The absolute categorical agreement of 91.1% (93.7% without *C. krusei*), with only 0.1% VME among the more than 10,000 isolates tested, easily meets the recognized criteria for a reliable surrogate marker as applied to antibacterial susceptibility testing (16). The use of fluconazole as a surrogate marker for posaconazole susceptibility was improved by designating those isolates for which the fluconazole MICs were ≤32 μg/ml (S and SDD categories combined) as susceptible to posaconazole, with the resistant category staying the same at ≥64 μg/ml. The resulting 97.6% categorical agreement and 0.2% VME rate are excellent for a surrogate marker test. Likewise, voriconazole was shown to perform well as a surrogate marker for posaconazole susceptibility and resistance, with a categorical agreement of 97.7% and a 0.3% VME rate. Thus, either fluconazole or voriconazole can be used effectively as a surrogate marker for posaconazole. The somewhat greater availability of fluconazole as a test reagent on commercial MIC panels (34, 38), and the ability of fluconazole to serve as a surrogate marker for both posaconazole and voriconazole (35), may make this agent a more convenient and useful tool than voriconazole for microbiology laboratories.

In conclusion, we have demonstrated the existence of cross-resistance among fluconazole, voriconazole, and posaconazole with the greatest emphasis on *C. glabrata*. Furthermore, we have shown that the availability of posaconazole susceptibility testing results for *Candida* spp., in any medical center currently performing antifungal susceptibility testing of either fluconazole or voriconazole, can be accomplished by using the fluconazole or voriconazole result as a surrogate marker for posaconazole susceptibility and resistance. Arguably, the most important role of in vitro susceptibility testing is to predict the resistance of the infecting organism to the agent under consideration for use in the patient (32, 33, 37). The occurrence of false-resistance errors with this application of the "class representative" concept to the available triazoles was very low and was acceptable for surrogate marker testing. Notably, only 15% of the fluconazole-resistant isolates (minus *C. krusei*) and 4% of the voriconazole-resistant isolates were susceptible to posaconazole at an MIC of ≤1 μg/ml. As commercial FDA-approved posaconazole susceptibility products become available, they should replace the interim use of surrogate markers for clinical testing. Until that time, microbiology laboratories may find it most convenient to use fluconazole as the surrogate marker for both voriconazole and posaconazole.

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